Claims

1. A method for preparing a template switched product encoded by at least part of one first template and at least part of at least one second template, wherein said product comprises at least one predetermined property, said method comprising the steps of

   i) providing a first template molecule and at least one second template molecule; and

   ii) providing a nucleic acid polymerase; and

   iii) synthesising a plurality of different template switched products by contacting sequentially in any order, or simultaneously, at least part of the first template and at least part of the at least one second template with said polymerase under conditions allowing for template dependent nucleotide polymerisation,

   wherein the synthesis of each individual template switched product involves at least one template switch,

   and wherein the synthesis of the plurality of different template switched products involves a plurality of template switches,

   iv) separating at least one template switched product comprising the at least one predetermined property from said plurality of template switched products; and

   v) obtaining a template switched product comprising at least one predetermined property

2. The method of claim 1, wherein

i) the first template comprises a first activity or encodes a molecule comprising a first activity; and

ii) the second template comprises a second activity or encodes a molecule comprising a second activity; and

iii) the predetermined property of the template switched product is a third activity, either comprised within the template switched product or in a molecule encoded by the template switched product; and

wherein the first and second activities are both different from the third activity.

3. The method of any of claims 1 and 2, wherein the method involves switches between more than 2 templates, such as 3, for example 4, such as 5, for example in the range from 5 to 10, such as from 10 to 20, for example from 20 to 50, such as from 50 to 100, for example from 100 to 200, such as more than 200 templates.

4. The method of claim 3, wherein the method involves more than 2 different template switches, such as 3, for example 4, such as 5, for example in the range from 5 to 10, such as from 10 to 20, for example from 20 to 50, such as from 50 to 100, for example from 100 to 200, such as more than 200 different template switches.

5. The method of claim 3, wherein the number of different template switched products generated is more than 20, for example more than 50, such as more than 500, for example more than 1000, such as more than 10000, for example more than 100000, such as more than 1000000.

6. The method of claim 3, wherein the number of different template switched products generated is in the range of from 1 to 1000000, such as from 1 to 500000, for example from 5 to 500000, such as from 5 to 250000, for example from 10 to 200000, such as from 10 to 150000, for example from 15 to 100000, such as from 5 to 250000, for example from 10 to 200000, such as from 10 to 150000, for example from 15 to 100000, such as from 20 to 200000, for example from 20 to 150000, such as from 25 to 150000, for example from 25 to 125000,

such as from 25 to 100000, for example from 50 to 100000, such as from 50 to 50000, for example from 100 to 50000.

7. The method of any of claims 1 to 6, wherein the nucleic acid polymerase comprises a RNase H activity.

8. The method of any of claims 1 to 7, wherein said method involves providing in a single reaction mixture more than 10 different templates and obtaining more than 100 different template switched products.

9. The method of any of claims 1 to 8, wherein the polymerase comprises reverse transcriptase activity.

10. The method of claim 1, wherein the method does not involve annealing between the first template and the template switched product.

11. The method of claim 1, wherein the method does not involve annealing between the second template and the template switched product.

12. The method of claim 1, wherein the method does involve annealing between the first template and the template switched product.

13. The method of claim 1, wherein the method does involve annealing between the second template and the template switched product.

14. The method of claim 1, wherein the first template and/or the second template are nucleic acid molecules.

15. The method of claim 14, wherein the first template and/or the second template encodes a polymer.

16. The method of claim 14, wherein the first template and/or the second template encodes a polypeptide.

17. The method of claim 15, wherein the first template and/or the second template encodes a scaffolded molecule.

18. The method of claim 1, wherein the template switched product is a nucleic acid.

19. The method of claim 18, wherein the template switched product encodes a polymer.

20. The method of claim 18, wherein the template switched product encodes a polypeptide.

21. The method of claim 18, wherein the template switched product encodes a scaffolded molecule.

22. The method of claim 1, wherein the first template and/or the second template are RNA molecules.

23. The method of claim 22, wherein the RNA molecule has been synthesised by an in vitro transcription reaction.

24. The method of claim 22, wherein the RNA molecule has been chemically synthesised.

25. The method of claim 22, wherein the RNA molecule has been purified from a cell extract.

26. The method of claim 1, wherein the method furthermore comprises introducing into said first and/or second template one or more template switch signals prior to synthesis of the template switched product.

27. The method of claim 26, wherein the template switch signal is a breakage in the template.

28. The method of claim 26, wherein the template switch signal comprises a predetermined secondary structure.

29. The method of claim 28, wherein the predetermined secondary structure comprises a double stranded nucleic acid .

30. The method of claim 28, wherein the predetermined secondary structure comprises a double stranded RNA.

31. The method of claim 28, wherein the predetermined secondary structure comprises a double stranded DNA

32. The method of claim 28, wherein the predetermined secondary structure comprises a double stranded nucleic acid, wherein one of the strand is selected from the group consisting of LNA and PNA.

33. The method of claim 26, wherein the template switch signal comprises a nucleotide analogue.

34. The method of claim 33, wherein the nucleotide analogue is capable of entering the active site of the polymerase, but not capable of being incorporated into a nucleic acid.

35. The method of claim 33, wherein the nucleotide analogue is capable of being incorporated to the end of a nucleic acid molecule, and wherein incorporation of the nucleotide analogue inhibits elongation of the nucleic acid.

36. The method of claim 33, wherein the nucleotide analogue is a phosphorothioate ribonucleotide analogue.

37. The method of claim 36, wherein the method comprises treatment of the first and/or second template with iodine.

38. The method of claim 27, wherein the nick(s) are introduced by limited enzymatic digestion.

39. The method of claim 38, wherein the enzymatic digestion is performed by a ribonuclease.

40. The method of claim 27, wherein the breakage is introduced by limited alkaline hydrolysis.

41. The method of claim 27, wherein the breakage is introduced by limited fragmentation using hydroxyl radicals.

42. The method of claim 1, wherein the synthesis comprises addition of one or more factors capable of affecting frequency and/or degree and/or accuracy of template switching.

43. The method of claim 42, wherein said factor is selected from the group consisting of DMS, kethoxal, CMCT, and DEPC.

44. The method of claim 42, wherein said factor is selected from the group consisting of a cationic detergent such as cetyltrimethylammonium bromide (CTAB), dodecyltrimethylammonium bromide (DTAB), tetramethylammonium chloride (TMAC), ions such as Na(+) and/or K(+) ions, and polymers such as polyethylene glycol (PEG), dextran sulphate, and polyvinylpyrrolidone.

45. The method of claim 42, wherein the factor is a protein.

46. The method of claim 45, wherein the protein is the nucleocapsid (NC) protein.

47. The method of claim 1, wherein the synthesis is performed at a temperature in the range of 0 to 5°C, such as 5-15°C, for example 15-25°C, such as 25-50°C, for example 50-70°C.

48. The method of claim 1, wherein the synthesis is performed in the presence of a divalent cation at a concentration in the range of from 6 mM to 25 mM, for example from 6 to 10 mM, such as from 10 to 20 mM.

49. The method of claim 1, wherein the synthesis is performed in the presence of dNTPs at a concentration in the range of from 1 μM to 100 μM, for example from 50 μM to 100 μM, such as from 10 μM to 75 μM, for example from 20 μM to 50 μM.

50. The method of claim 1, wherein said first and said second template molecule share in the range of 10 to 99% identity.

51. The method of claim 1, wherein a region comprising 5 nucleotides in said first template share at least 40%, such as at least 60%, for example at least 80% identity with a region comprising 5 nucleotides in the second template..

52. The method of claim 1, wherein said first and said second template molecule share in the range of 50 to 95% identity.

53. The method of claim 1, wherein one or more templates have been prepared by a method comprising the steps of

i) Providing a DNA molecule; and
ii) Providing a mixture of nucleotides and nucleotide analogues, wherein one or more of said nucleotide analogues once incorporated into a nucleic acid do not allow further elongation; and
iii) Contacting said DNA molecule with said mixture and transcribing and/or replicating said DNA; and
iv) thereby obtaining one or more different templates.

54. The method of claim 1, wherein one or more templates have been prepared by a method comprising the steps of

i) providing a RNA molecule; and

ii) fragmenting said RNA molecule into RNA fragments; and

iii) replicating one or more of said RNA fragments; and

iv) thereby obtaining one or more different templates

55. The method of claim 53, wherein the nucleotide analogues are selected from the group of dexoyribonucleotide analogs

56. The method of claim 53, wherein the nucleotide analogues are selected from the group of ribonucleotide analogs.

57. The method of claim 1, wherein the method comprises amplification of the plurality of template switched products.

58. The method of claim 57, wherein said amplification involves a PCR reaction.

59. The method of claim 1, wherein the sequence of steps are repeated at least once.

60. The method of claim 59, wherein template switched product(s) are used as a first template and/or second template when the method is repeated.

61. Use of the method of any of claims 1 to 60 for gene shuffling.